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NITROGENOUS COMPONENTS OF NUTRIENT MEDIUM FOR CULTIVATION OF CLOSTRIDIUM PERFRINGENS TYPE D

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NITROGENOUS COMPONENTS OF NUTRIENT MEDIUM FOR CULTIVATION OF CLOSTRIDIUM PERFRINGENS TYPE D

by

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NITROGENOUS COMPONENTS OF NUTRIENT MEDIUM FOR CULTIVATION OF CLOSTRIDIUM PERFRINGENS TYPE D

A growing microbe cell is closely related with the nutrient medium in which it is cultivated. From the medium the cell obtains the substances necessary for its development; the cell also releases the by- products of its metabolism to the medium.

We studied the dynamics of the nitrogenous components of the medium in the growth and toxin-formation of Cl. perfringens strains of different toxicity. The results of this research can have significance for the study of the metabolism of a microbe cell and its relation to toxin formation.

MATERIAL AND METHODS: The work was conducted on two strains obtained from the Microbiology and Immunity Laboratory of the All-Union Institute of Experimental Veterinary Medicine (VIEV): typical toxigenic strain 91 and strain 47 which had lost its ability to produce epsilon-toxin, the basic toxin of type D. Both strains were similar in their morphological, cultural, and some of their biochemical properties (lecithinase and hemolytic activity).

The microorganisms were grown on a casein-fungus Vinogradov medium obtained from the N. F. Gamalei Institute of Epidemiology and Microbiology; 50 ml of 18 - 20 hour culture grown on a casein-fungus medium was sown in 2.5 l. of Vinogradov medium. Samples were taken after 1.5, 3, 6, 8, 12, 18, 24, and 48 hours, passed through a cotton filter, and liberated from the microbe mass by centrifuging at 3.5 to 4 thousand rpm. The number of microbes was determined by optical density on a FEK-M instrument.

The lethal properties of the toxin were studied by means of interabdominal introduction of a dose of 0.5 ml native and trypsinized toxin into white mice weighing 16 to 18 grams; the lecithinase and hemolytic properties were studied by the method described by F. M. Tyutnikov (1965).

Biochemical investigation of the following indices of the media and filtrates of the cultures of different growth periods were conducted: total and residual nitrogen after settling out of the protein by 21% solution of trichloroacetic acid was determined by the colorimetric method with a Nessler reagent; the protein nitrogen was determined by the difference between the total and residual nitrogen; the amino-nitrogen by the Harting and Maclean method as modified by N. M. Klimov, V. V. Sukhov, and Ye. Sh. Zhuravel' (1966). In all, five series of tests were conducted.

RESULTS OF THE INVESTIGATION: In the analysis of the data received by us, it should be noted that the beginning of toxin-formation coincided with the beginning of cell division (three-hour culture). The most lethal, hemolytic, and lecithinase activity of the culture filtrates was observed after 12 - 24 hours of growth.

In comparison with strain 91, the growth of the bacterial mass in strain 47 was retarded beginning with the eighth hour of culture growth (the number of microbes in strain 47 after 8 hours was 0.85 billion/ml, and in strain 91, 1.04 billion/ml; after 48 hours the respective quantities were 1.33 and 2.24).

We also determined the regularity in variations of particular nitrogen components of the medium in the growth of both strains. Thus in the culturing of both strains for 1.5 to 48 hours, the total nitrogen (10.8--13.01 mg/ml) and protein (3.95--6.18 mg/ml) in the filtrates of the cultures was discovered to be less, while the residual (6.22--6.94 mg/ml) and amino-nitrogen (1.45--3.31 mg/ml) was discovered to be greater than in a sterile medium (the total nitrogen in the medium was 13.3 mg/ml, the residual nitrogen was 6.19, the protein nitrogen was 7.13, and the amino-nitrogen was 1.28 mg/ml).

Some strain differences were noted in the variations of the medium. In the cultivation period of strain 47 from 6 to 18 hours, there was a gradual reduction in the level of protein nitrogen to 3.95 mg/ml; in the same period of strain 91 growth variations in its level were noted (in 6, 12, and 18 hour cultures there was an increase to 6.05--6.18 mg/ml).

The quantity of amino-nitrogen in the initial period of culturing in both strains was decreased (in a three hour culture it reached the lowest level: 1.45--1.48 mg/ml). Then in strain 91 the content of amino-nitrogen sharply increased and was especially pronounced in an eight your culture (3.31 mg/ml). In the growth of strain 47, the level of amino-nitrogen was stable (1.51--1.81 mg/ml).

Attention is directed to the relatively constant level of residual nitrogen in both strains (6.22--6.94 mg/ml, excluding six hour culture of strain 91, which was 5.94 mg/ml, and eight hour culture of strain 47, which was 7-30 mg/ml); the pH of the medium shifted to the acid side in the growth of both strains (7.7--6.73).

It is necessary to assume that such media variations are related to the proteolytic activity of the microbe cell. The proteolytic ferments of Cl. perfringens type A were investigated by V. A. Blagoveshchenskiy and others (1964). The authors discovered strengthening of the proteolytic activity in the toxigenic strain. Our data about the increase in amino-nitrogen directly confirms this position.

Some of the increase of the total and protein nitrogen in the growth of a toxigenic strain in the period of intensive growth and toxin formation is apparently related to the passage into the medium of toxin of a protein nature.

CONCLUSIONS

1. Toxin formation in Cl. perfringens type D is related to the multiplication of the culture.

2. In culturing of toxigenic and weak-toxigenic strains of Cl. perfringens type D on a semi-synthetic medium based on a hybrid hydrolysate of casein, the level of total and protein nitrogen of the culture filtrates diminished, while the residual and amino-nitrogen increased in comparison with a sterile medium.

3. In the growth of a toxigenic strain there is a sharp increase in the quantity of amino-nitrogen in the medium during the period of intensive growth and toxin formation. In the filtrates of a culture of a weak-toxigenic strain, the level of nitrogen during the whole period of culturing was relatively stable. This regularity is related to the increased proteolytic activity of the toxigenic strain.

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13. ABSTRACT

This article concerns the dynamics of the nitrogenous components of the medium in the growth and toxin-formation of Clostridium perfringens of different toxicity.

From their research, the authors made the following conclusions: (1) toxin formation in Cl-perfringens type D is related to the multiplication of the culture; (2) in culturing of toxigenic and weak-toxigenic strains of Cl-perfringens type D on a semi-synthetic medium based on a hybrid hydrolysate of casein, the level of total end protein nitrogen of the culture filtrates diminished, while the residual and amino-nitrogen increased in comparison with a sterile medium; (3) in the growth of a toxigenic strain there is a sharp increase in the quality of amino nitrogen in the medium during the period of intensive growth and toxin formation. In the filtrates of a culture of a weak-toxigenic strain, the level of nitrogen during the whole period of culturing was relatively stable. This regularity is related to the increased proteolytic activity of the toxigenic strain.

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